PNAS
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Supplementary Information for

VLM Catecholaminergic Neurons Control Tumor Growth by Regulating CD8$^+$ T Cells

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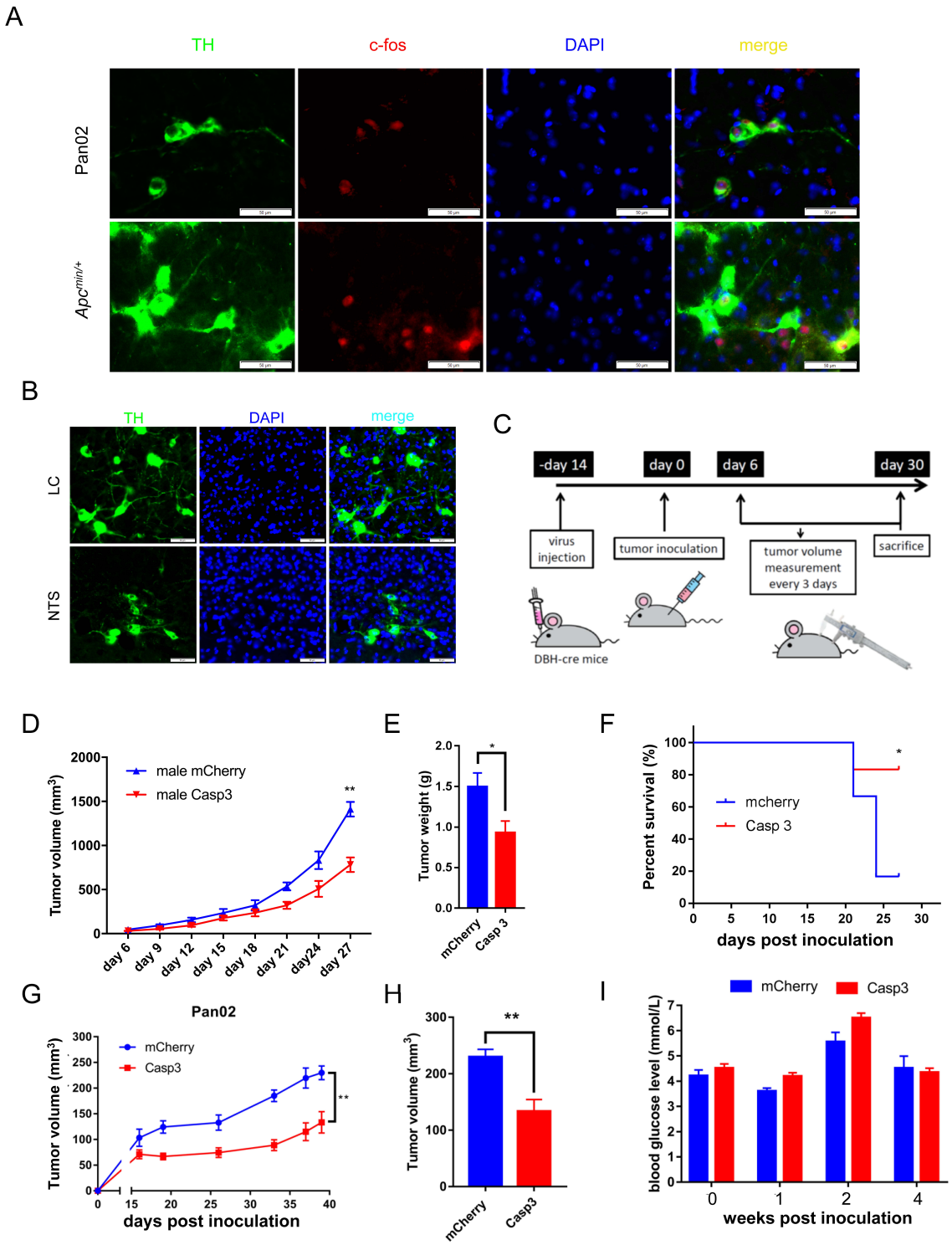

Figure S1. Syngeneic mouse tumors activate VLM catecholaminergic neurons (VLM CA neurons), and depletion of VLM CA neurons slow down tumor growth. (A) 1×10^6 Pan02 mouse pancreatic ductal cells were inoculation into the flanks of C57BL/6 mice or *Apc*$^{min/+}$ spontaneous mice alone, without tumor cell line inoculation. All of them showed activation after tumor inoculation or induction. TH and c-Fos double positive cell number in VLM CA: 183±11 (Pan02) vs 247±9 (*Apc*$^{min/+}$). (B) Virus injection did not affect other brain regions. After introducing the AAV-Casp3 into the region of VLM CA, the mouse brains were sectioned and the

TH staining was conducted in LC and NTS regions. LC: Locus Coeruleus; NTS: nucleus tractus solitarius. TH positive cells were detected with the number: 1897±126 (LC) vs 1229±176 (NTS) that are similar to the previous report (11). (C) Schematic for establishment of the animal model. Mice were injected with the indicated AAV virus two weeks before tumor inoculation, and from day 6, we measured tumor volume using digital calipers until day 30. (D) Ablation of VLM CA neurons by AAV-taCasp3 in male mice can slow syngeneic tumor growth. (n_{Casp3}=4, $n_{mCherry}$=4). (E) Ablation of VLM CA neurons can reduce tumor weight. (n_{Casp3}=4, $n_{mCherry}$=4). (F) Ablation of VLM CA neurons can extend the survival of male mice (n_{Casp3}=6, $n_{mCherry}$=6). (G-H) The ablation of VLM CA neurons can also slow down Panc02 inoculation tumor growth (n_{Casp3}=9, $n_{mCherry}$=5). (I) There was no difference in the blood glucose level between AAV-Casp3 and AAV-mCherry mice (n_{Casp3}=11, $n_{mCherry}$=9).
Results shown represent the mean±SEM. *p<0.05, **p<0.01, ***p<0.001.

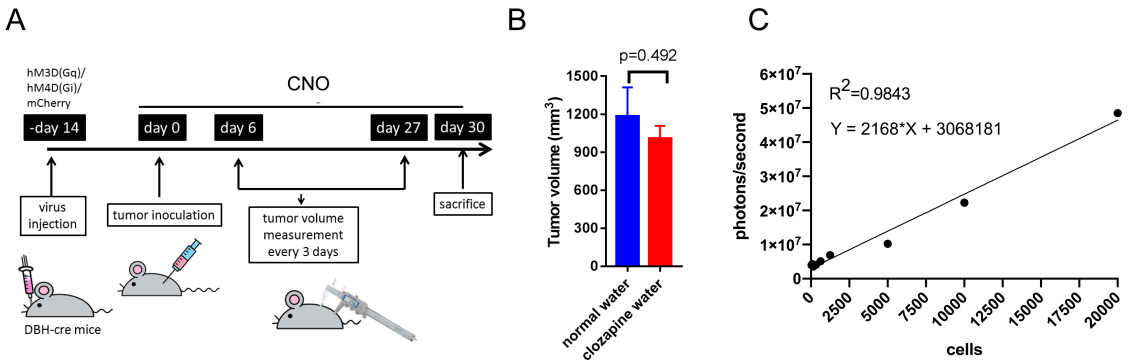

Figure S2. The activity of VLM CA neurons controls tumor growth. (A) Schematic for the establishment of the animal model. (B) CNO did not have influence on tumor growth ($n_{normal\ water}$=8, $n_{CNO\ water}$=8). (C) IVIS system; luciferase activity was measured using 2-fold serial dilutions of luciferase-tagged MC-38 cancer cells (MC38-luc).
Results shown represent the mean±SEM. *p<0.05, **p<0.01, ***p<0.001. Results are pooled from two or three independent experiments.

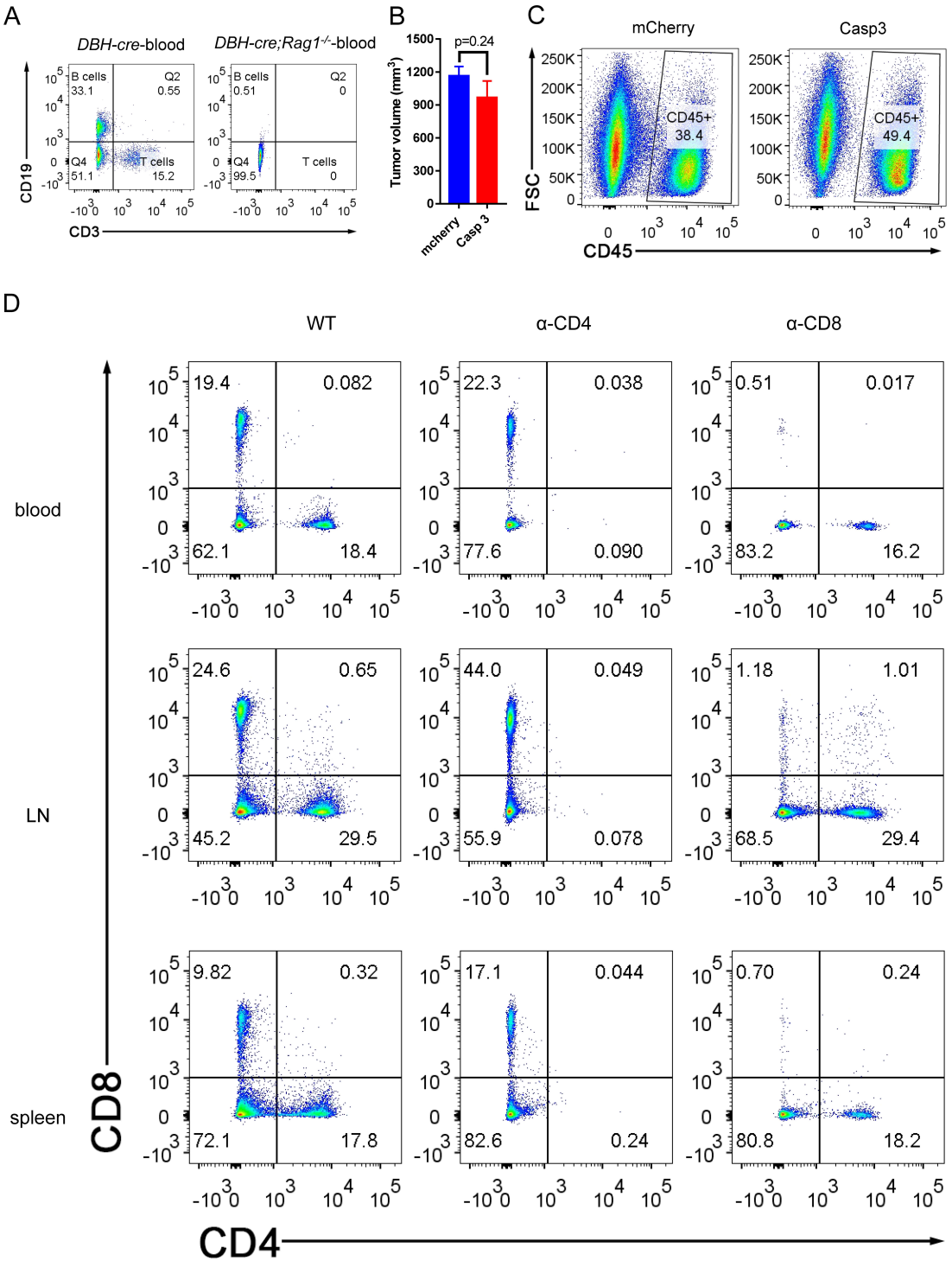

Figure S3. The adaptative immune system functions in the VLM CA regulated tumor growth. (A) FACS results showing that there were no T lymphocytes (neither $CD4^+$ nor $CD8^+$ T cells) or B lymphocytes in *Dbh-Cre;Rag1*$^{-/-}$ mice. (B) No differences in tumor volume were observed upon ablation of VLM CA neurons from *Dbh-Cre; Rag1*$^{-/-}$ mice (n_{Casp3}=8, $n_{mCherry}$=14). (C) FACS results showing increased $CD45^+$ leucocyte infiltration into tumors upon ablation of VLM CA neurons. (D) FACS results showing successful selective depletion of $CD4^+$ or $CD8^+$ T cells based on $CD4^+$ or $CD8^+$ depletion antibodies. LN, lymph node.

Results represent the mean±SEM. *p<0.05, **p<0.01, ***p<0.001. Results are pooled from two or three independent experiments.

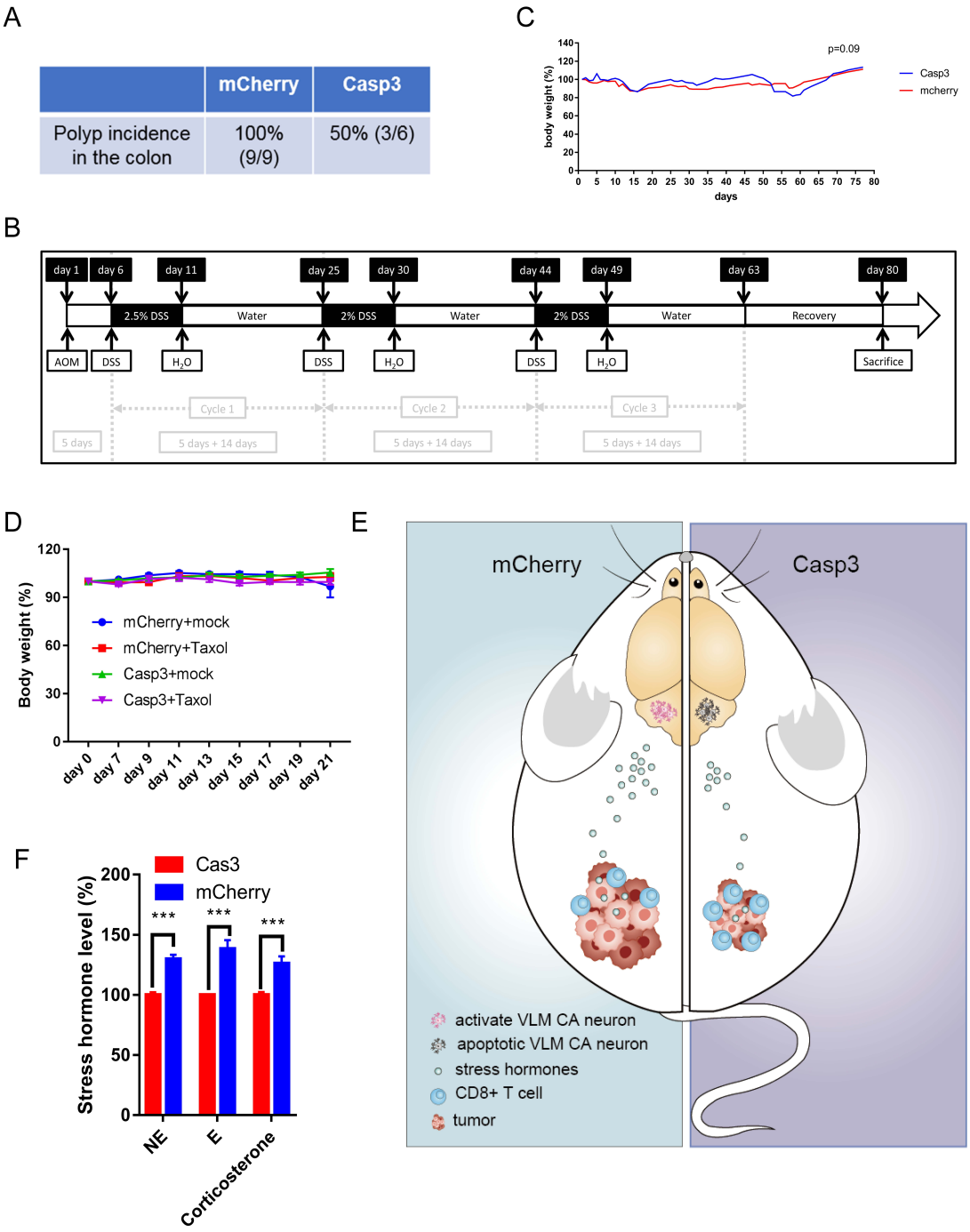

Figure S4. VLM CA neuron ablation slows down spontaneously tumor progression. (A) There was reduced polyp incidence in the colon after VLM CA neurons ablation. (B) Schematic view of AOM/DSS model establishment. (C) The body weight of AAV-mCherry and AAV-Casp3 mice. There was slightly heavy body weight after VLM CA neurons ablation in the AOM/DSS mouse model. (D) There were no side effects of taxol in mice indicated by body weight. (E) Schematic model summarization of VLM CA neurons regulation in tumor progression. (F) NE, N and corticosterone levels in mice serum. Normalized by Casp3 group.
Results shown represent the mean±SEM. *p<0.05, **p<0.01, ***p<0.001.